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REMARKS/ARGUMENTS

The foregoing amendments in the specification are of formal nature and do not add new matter. The newly introduced claims are fully supported by the specification as originally filed, such as, for example, at page 3, lines 16 - 38; Figures 1A-B; in the passage bridging pages 8 and 9; Example 1; and page 27, line 1 to page 29, line 14. Reviewing the Sequence Listing of record, applicants found that it included an incorrect sequence as SEQ ID NO: 5. The enclosed substitute Sequence Listing includes the correct SEQ ID NO: 5, which is the amino acid sequence of murine GFR $\alpha$ 3. As the correct sequence was disclosed, e.g. at Figure 2 at the time of filing the application, the substitute Sequence Listing does not contain new matter.

Prior to entry of the present amendment, claims 1-15 were pending in this application. In an Office Action mailed on October 27, 2000 (Paper No. 20) all claims were rejected on various grounds. The present submission is accompanied by a request for a three months extension of time, setting the time for response to April 27, 2001.

Claim rejections - 35 U.S.C. § 101

Claims 13-14 were rejected as being directed to non-statutory subject matter. The Examiner noted that the recitation of "A host cell" encompasses a human organism. Claims 13-14 have been canceled. Newly introduced claims 78-81 recite an "isolated" host cell, which is believed to overcome this rejection.

Claims 1-15 were rejected for alleged lack of specific and/or substantial asserted utility or a well established utility supporting these claims. According to the rejection, at the time of filing the instant application GFR $\alpha$ 3 was an orphan receptor, therefore, "the claimed polynucleotides have no specific nor substantial utility because further experimentation is necessary at the time of filing the instant invention to attribute a function and 'real world' utility to the claimed nucleic acid molecules." Claims 1-15 are canceled. Newly added claims 66-85 are believed to cover subject matter which has patentable utility.

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Although at the time of filing the present application, the native ligand of GFR $\alpha$ 3 was unknown, the application is not without disclosure concerning the function of GFR $\alpha$ 3. According to the expression data disclosed in Example 5, in the mouse GFR $\alpha$ 3 mRNA was very strongly expressed in dorsal root ganglia, in sympathetic ganglia, and in peripheral nerves. The vestibular ganglion also displayed strong signal. At later developmental stages, expression within the CNS was very limited. Human GFR $\alpha$ 3 showed a similar expression pattern, which, unlike the related GFR $\alpha$ 1 and GFR $\alpha$ 2 receptors, was very limited and localized (see Figure 8). According to page 47, lines 26-27, "*In situ* hybridization studies using DNA encoding mouse GFR $\alpha$ 3 revealed a pattern of expression in peripheral sensory neurons and sympathetic neurons." Based on these data, at page 29, lines 2-8, the specification teaches that "GFR $\alpha$ 3 . . . can be used to treat conditions involving dysfunction of the autonomic nervous system including, but not limited to, disturbances in blood pressure or cardiac rhythm, gastrointestinal function, impotence, and urinary continence."

Furthermore, on page 28, lines 15-33, applicants disclose that "Non-human homologues of GFR $\alpha$ 3 can be used to construct a GFR $\alpha$ 3 "knock out" animal which has a defective or altered gene encoding GFR $\alpha$ 3 as a result of homologous recombination between the endogenous gene encoding GFR $\alpha$ 3 and altered genomic DNA encoding GFR $\alpha$ 3 introduced into an embryonic cell of the animals. . . . Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the GFR $\alpha$ 3 polypeptide.

The important role of GFR $\alpha$ 3 in the survival of peripheral neurons has been confirmed following the discovery of its native ligand, artemin (neublastin). Moreover, knock-out mice in which the GFR $\alpha$ 3 gene was disrupted, generated as taught in the present application, confirmed that GFR $\alpha$ 3 is required for migration and survival of the superior cervical ganglion. See Nishino *et al.*, *Neuron* 23:725-736 (1999), a copy of which is submitted with the Information Disclosure Statement filed concurrently herewith. Such transgenic mice could not have been produced and used without applicants' identification and disclosure of the nucleic acid sequence encoding murine GFR $\alpha$ 3.

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It is submitted that the teaching provided in the present application as filed, in view of subsequent confirmatory data, meets the utility requirement of 35 U.S.C. § 101. Accordingly, the reconsideration and withdrawal of the present rejection, as it might concern newly submitted claims 66-85, is respectfully requested.

*Claim Rejections - 35 U.S.C. § 112*

(1) Claims 1-15 were rejected under 35 U.S.C. § 112, first paragraph, as “since the claimed invention is not supported by either a specific and/or substantial asserted utility or a well established utility . . . one skilled in the art would not know how to use the claimed invention.” Claims 1-15 have been canceled. In the foregoing analysis, we have shown that the invention as claimed does have a specific and/or substantial asserted utility, accordingly, the present rejection, as it might apply to the claims currently pending, should also be withdrawn.

(2) Claims 1-15 were rejected under 35 U.S.C. § 112, first paragraph “as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.” In addition to the reasoning used in the “lack of utility” rejection, the Examiner noted that the name “nucleic acid having at least 65/75% sequence identity to a nucleic acid encoding a GFR $\alpha$ 3 polypeptide” does not sufficiently characterize and enable the polynucleotides that are encompassed by the claims.

The claims now recite isolated nucleic acid molecules that comprise a nucleic acid encoding a polypeptide having at least 80% sequence identity with amino acid residues 27 to 374 of the native sequence murine GFR $\alpha$ 3 polypeptide of Figures 1A-B (SEQ ID NO: 5), and have the ability to regulate peripheral neuronal function. While some experimentation might be necessary to identify the amino acid sequences variants of the native sequence which retain the ability to regulate peripheral neuronal function, a person skilled in the art at the time of filing this application knew how to make such variants and test their function, without undue experimentation. Accordingly, the withdrawal of this rejection, as it might apply to the new claims, is respectfully requested.

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(3) Claims 7-8 were rejected for alleged lack of enablement. According to the rejection, the "specification lacks sufficient deposit information for the cDNAs deposited with the ATCC." Claims 7-8 have been canceled, and the newly added claims do not refer to ATCC deposits. Accordingly, this rejection is believed to be moot.

(4) Claims 8 and 10 were rejected as "being indefinite" for failing to define the metes and bounds of "stringent [hybridization] conditions." Claims 8 and 10 have been canceled. As the newly added claims do not refer to "stringent [hybridization] conditions," this rejection is moot.

Claim rejections - 35 U.S.C. § 102

Claims 1-15 were rejected under 35 U.S.C. 102(a) as being anticipated by Sanicola-Nadel et al., WO 97/44356.

WO 97/44356 was published on November 27, 1997, i.e. less than a year before the earliest priority date (April 13, 1998) of the present application. Having reviewed the first three priority documents of WO 97/44356 (the fourth priority document not having been furnished), applicants found that they did not disclose the murine retL3 sequence (SEQ ID NO: 17), corresponding to the murine GFR $\alpha$ 3 sequence disclosed in the present application, and recited in the present claims. Accordingly, the earliest date by which this sequence might have been disclosed in WO 97/44356 is April 10, 1997. Applicants have conceived and reduced to practice, in the United States, the murine GFR $\alpha$ 3 nucleic acid before that date. A Declaration under 37 C.F.R. § 1.131 will be submitted shortly. As the cited reference did not describe the claimed invention before applicants' invention thereof, it does not anticipate the claims currently pending.

In conclusion, pending the filing of the signed Declaration under 37 C.F.R. §1.131, all claims pending in this application are believed to be in *prima facie* condition for allowance. Should the Examiner find that there are any issues outstanding, he is respectfully invited to contact the undersigned attorney at the telephone number indicated below.

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Attached hereto is a marked-up version of the changed made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: April 27, 2001

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

In the Specification:

The paragraph beginning at page 1, line 11, has been amended as follows:

--Neurotrophic factors such as insulin-like growth factors, nerve growth factors, brain-derived neurotrophic factor, neurotrophin-3, -4/5 and -6, ciliary neurotrophic factor, GDNF, and neurturin have been proposed as potential means for enhancing specific neuronal cell survival, for example, as a treatment for neurodegenerative diseases such as amyotrophic lateral sclerosis, Alzheimer's disease, stroke, epilepsy, Huntington's disease, Parkinson's disease, and peripheral neuropathy. It would be desirable to provide additional therapy for this purpose. Protein neurotrophic factors, or neurotrophins, which influence growth and development of the vertebrate system, are believed to play an important role in promoting the differentiation, survival, and function of diverse groups of neurons in the brain and periphery. Neurotrophic factors are believed to have important signaling functions in neural tissues, based in part upon the precedent established with nerve growth factor (NGF). NGF supports the survival of sympathetic, sensory, and basal forebrain neurons both *in vitro* and *in vivo*. Administration of exogenous NGF rescues neurons from cell death during development. Conversely, removal or sequestration of endogenous NGF by administration of anti-NGF antibodies promotes such cell death (Heumann, *J. Exp. Biol.*, 132:133-150 (1987); Hefti, *J. Neurosci.* 6:2155-2162 (1986); Thoenen, *et al.*, [*Annu Rev. Physiol.*] *Physiol. Rev.*, 60:[284-335] 1284-1335 (1980)).--

The paragraph beginning at page 1, line 25 has been amended as follows:

-- Additional neurotrophic factors related to NGF have since been identified. These include brain-derived neurotrophic factor (BDNF) (Leibrock, *et al.*, *Nature*, 341:149-152 (1989)); neurotrophin-3 (NT-3) (Kaisho, *et al.*, *FEBS Lett.*, 266:187 (1990); Maisonpierre, *et al.*, *Science*, 247:1446 (1990); Rosenthal, *et al.*, *Neuron*, 4:767 (1990), and neurotrophin 4/5 (NT-4/5) ([Berkmeier] Berkemeier, *et al.*, *Neuron*, 7:857-866 (1991)).--

The paragraph beginning at page 17, line 33 and ending at page 18, line has been amended as follows:

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-- Glial cell line-derived neurotrophic factors ("GDNF") (Lin *et al.*, *Science*, 260:1130-1132 (1993); WO 93/06116, which are incorporated herein in its entirety), is a potent survival factor for midbrain dopaminergic (Lin *et al.*, [Science, 260:1130-1132] (1993), *supra*; Strömberg *et al.*, *Exp. Neurol.*, 124:401-412 (1993); Beck *et al.*, *Nature*, 373:339-341 (1995); Kearns *et al.*, *Brain Res.*, 672:104-111 (1995); Tomac *et al.*, *Nature*, 373:335-339 (1995)), spinal motor (Henderson *et al.*, *Science*, 266:1062-1064 (1994); Oppenheim *et al.*, *Nature*, 373:344-346 (1995); Yan *et al.*, *Nature*, 373:341-344 (1995)), and noradrenergic neurons (Arenas *et al.*, *Neuron*, 15:1465-1473 (1995)), which degenerate in Parkinson's disease (Hirsch *et al.*, *Nature*, 334:345-348 (1988); Hornykiewicz, *Mt. Sinai J. Med.*, 55:11-20 (1988)), amyotrophic lateral sclerosis (Hirano, *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disease*, P. Rowland, ed. (New York: Raven Press, Inc.) pp. 91-101 (1991), and Alzheimer's disease ([Marcyniuk] *Marcyniuk et al.*, *J. Neurol. Sci.*, 76:335-345 (1986); Cash *et al.*, *Neurology*, 37:42-46 (1987); Chan-Palay *et al.*, *Comp. Neurol.*, 287:373-392 (1989)), respectively. Based on mice genetically engineered to lack GDNF, additional biological roles for GDNF have been reported: the development and/or survival of enteric, sympathetic, and sensory neurons and the renal system, but not for catecholaminergic neurons in the central nervous system (CNS) (Moore *et al.*, *Nature* 382:76-79 (1996); Pichel *et al.*, *Nature* 382:73-76 (1996); Sanchez *et al.*, *Nature* 382:70-73 (1996)). Despite the physiological and clinical importance of GDNF, little is known about its mechanism of action.--

The paragraph beginning at page 51, line 21 has been amended as follows:

-- Primers containing sense sequence GCCCGCGACCTCCACTGCTG (designated gfrp1; SEQ ID NO: 22) and [antisense] antisense sequence CTGTGGGGAGCGGCGGCG (designated gfrp2.r.c; SEQ ID NO: 23) were used to generate a 671 bp hybridization probe from the mouse GFR $\alpha$ 3. Primers containing sense sequence CCTGAACCTATGGTAACTGG (SEQ ID NO: 24) and antisense sequence ACCCAGTCCTCCCTACC (SEQ ID NO: 25) were used to generate a 378 bp hybridization probe from the mouse GFR $\alpha$ 3.--

The paragraph beginning at page 53, line 10 has been amended as follows:

-- In an alternative technique, mammalian GFR $\alpha$ 3 may be introduced into 293 cells transiently using the dextran sulfate method described by [Somparyac] Sompayrac et al., *Proc.*

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*Natl. Acad. Sci.*, [12] 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-*GFRα3* DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20 % glycerol for 90 second, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µh/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed mammalian *GFRα3* can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography. --

The paragraph beginning at page 54, line 17 has been amended as follows:

--Alternatively, expressed poly-his tagged *FGRα3* can be purified by  $\text{Ni}^{2+}$ -chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by [Rupert] Ruppert *et al.*, *Nature* 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM  $\text{MgCl}_2$ ; 0.1 mM EDTA; 10% Glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50mM phosphate, 300 mM NaCl, 10% Glycerol, pH 7.8) and filtered through a 0.45 µm filter. A  $\text{Ni}^{2+}$ -NTA agararose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to a baseline  $A_{280}$  with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50mM phosphate; 300 mM NaCl, 10% Glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching  $A_{280}$  baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. On mL fractions are collected and analyzed by SDS-PAGE and silver staining or western blot with  $\text{Ni}^{2+}$ -NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged *GFRα3* are pooled and dialyzed against loading buffer.--



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Please cancel the Sequence Listing numbered as pages 1-34 immediately following the Abstract of the Disclosure, and replace it with the attached substitute Sequence Listing, pages 1-26.

In the Claims:

Claims 1-15 have been canceled and new claims 66-85 have been added.

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